NEURODEGENERATION

Parkin function in Parkinson's disease

Models of Parkin-mediated ubiquitination lends insight into the role of pathological mutations

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Parkinson's disease (PD) is the second most common neurodegenerative disease, and is characterized by involuntary shaking, muscle rigidity, and the progressive loss of dopaminergic neurons. In ~5 to 10% of PD cases there is a genetic association, with almost 20 genes attributed to date. One example is early-onset autosomal recessive (AR) PD, for which the majority of cases are linked to mutations in the Parkin gene (*PRKN*; also known as *PARK2*). *PRKN* encodes the E3 ubiquitin ligase Parkin, which plays important roles in mitochondrial quality control and turnover. Parkin, although localized to the mitochondria under certain conditions, is primarily cytosolic (*1*). A second ARPD-associated gene, *PINK1* (PTEN-induced putative kinase 1), encodes a mitochondrially tethered kinase that regulates Parkin activity through phosphorylation events. Mutations in *PINK1*, although rare, are associated with a phenotype similar to that of ARPD patients with *PRKN* mutations. Numerous mutations throughout *PRKN* are linked to ARPD, making the functional examination of Parkin crucial to understanding ARPD pathogenesis. A wealth of structural studies have transformed our knowledge of Parkin regulation and catalytic mechanisms. However, the current picture is incomplete, leading to several possible models of Parkin catalysis, which has implications for understanding how the ARPD-associated mutations affect the protein and thus PD pathogenesis.

The major function of Parkin is to ligate ubiquitin to lysine residues, an essential post-translational modification involved in almost every cellular pathway. Ubiquitination occurs via the sequential action of three enzymes: an E1-activating enzyme, an E2-conjugating enzyme, and an E3 ligase. Parkin belongs to the RBR [RING–in-between-RING (IBR)–RING] family of E3 ligases, which contain a RING1 domain for E2 recruitment, a catalytic domain (RING2) through which transfer of ubiquitin is mediated, and a linking IBR domain (*2*). There are 14 RBR family members, including HOIP (HOIL-1–interacting protein), HHARI (human homolog of Ariadne), and Dorfin (double ring-finger). Owing to its relevance in ARPD, Parkin is the most studied RBR ligase and thus also serves as an important model for understanding RBR mechanism.

Parkin comprises five domains: ubiquitin-like domain (Ubl), RING0, RING1, IBR, and RING2. The Ubl and RING0 domains are unique to Parkin, with the RBR module common to all RBR ligases. ARPD-associated *PRKN* mutations are distributed across all five domains and are also found within domain-domain interfaces and linker regions. This broad distribution of mutations across all of Parkin suggests the structural integrity of all domains is important in ARPD pathogenesis. Parkin adopts an autoinhibited conformation mediated by its amino-terminal Ubl domain (*3*). The Ubl is joined to the carboxyl-terminal RING0-RBR (R0RBR) domains by a flexible 65–amino acid linker. Crystal structures of R0RBR Parkin, with both the Ubl and linker removed, reveal a rigid core formed of RING0, RING1, and RING2 domains, with the IBR domain showing degrees of flexibility (*4, 5*). Structures of Parkin that include the Ubl domain show that the Ubl is associated with RING1 (*4*, *6*, *7*). Each structure highlights several autoinhibitory features: The RING0 partially occludes the catalytic cysteine (Cys^{431}); a small helical element [repressor element of Parkin (REP)] along with the Ubl domain blocks the predicted E2 binding site on RING1; and finally, the distance between the RING2 Cys^{431} and predicted E2 catalytic cysteine is over 30 Å, which is too far to allow transfer of ubiquitin. Thus, for full Parkin function, not only are activators required to disrupt the autoinhibitory mechanisms, but large conformational changes are predicted to bridge the distance between catalytic sites.

Multiple ARPD-associated proteins such as Parkin, PINK1, and DJ-1 (also known as PARK7), play roles in mitochondrial homeostasis, suggesting that dysfunction in this process may be a feature of ARPD. PINK1 phosphorylates Parkin at Ser^{65} of the Ubl domain, which increases its ubiquitin ligase activity (*1*). In addition to directly phosphorylating Parkin (pParkin), PINK1 phosphorylates ubiquitin at the equivalent Ser^{65} (pUb), which leads to activation of Parkin (*1*). Allosteric binding of pUb to Parkin weakens Ubl association with the R0RBR domains in trans (*6-8*), and results in straightening of helix 3 within RING1 to facilitate movement of the IBR domain (*8, 9*). Furthermore, PINK1 phosphorylates Parkin-pUb more efficiently than Parkin alone, suggesting that displacement of the Ubl is required for full kinase activity (*10*). In pParkin, the pUbl is released to form an extended conformation, and only in the presence of pUb (*11*). Point mutations that disrupt individual autoinhibitory features of Parkin show that upon activation with pUb, Parkin undergoes conformational changes that release both REP and the Ubl domain (*12*).

E2s transfer ubiquitin to Parkin, yet interaction between E2 and autoinhibited Parkin is weak (*6*). However, phosphorylation of Parkin and ubiquitin renders the E2 interaction measurable (*6, 7*). Although the interaction of pParkin-pUb and E2s is low, it is increased when the E2 is loaded with ubiquitin (E2~Ub) (*7*). Finally, computational analyses predict that Ubl phosphorylation initiates an unwinding of domains (*13*). Thus, the prevailing view of PINK1-mediated Parkin activation is one where both phosphorylation events are needed to drive conformational changes to release the Ubl domain, the REP, and potentially, the RING0 domain to allow binding of E2~Ub and ubiquitin transfer. However, the structure of pUb bound to the R0RBR domains shows no movement of the REP (*8*). Furthermore, a recent crystal structure of all five human Parkin domains (UblR0RBR) bound to pUb shows no displacement of the Ubl domain or the REP, nor alteration of the catalytic cysteine environment (*9*). One caveat is that the Ubl-RING0 linker is missing. This linker is not conserved in Parkin orthologs, but does influence in vitro activity (*7*). Furthermore, Parkin is not phosphorylated in this structure and there is no E2~Ub present. The straightening of RING1 helix 3 observed in the pUb-R0RBR structure is also observed in the presence of the Ubl domain (*8, 9*). In this context, however, the IBR is displaced, revealing a hidden ubiquitin binding site on RING1 helix 3 that is proposed to interact with the donor ubiquitin from E2~Ub. Thus, pUb and ubiquitin bind on opposing sides of the now-straightened RING1 helix 3, and mutation of either face leads to reduced affinity for pUb as well as E2~Ub (*13, 14*).

This bipartite binding of ubiquitin molecules is also observed in the crystal structure of another RBR ligase, HOIP, where ubiquitin binds in the equivalent position as pUb, and E2~Ub binding corresponds to the hidden ubiquitin binding site (*14*). Both of these structures

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show that the catalytic cysteine and the donor ubiquitin are still too far for ubiquitin transfer from E2 to Cys^{431} in cis. Does the E2 to RING2 transfer of ubiquitin require further conformational changes of the RBR modules, or are other mechanisms possible? This conundrum is reminiscent of the allegory of the long spoons where in Hell, the inability to feed oneself with such unwieldly tools is circumvented in Heaven by feeding one another. Interestingly, self-association of E3 molecules has been reported to support Parkin activity both in vitro and in cells (*15*), thus raising the possibility of trans-cooperation for catalytic function (see the figure). Further support for this comes from the crystal structure of HOIP RBR-E2~Ub complex, where the RING2 of a neighboring molecule is in proximity to E2~Ub bound to a different RBR module (*14*). This "domain-swapped" dimer suggests the possibility of a trans-based mechanism. Intriguingly HOIP functions biologically in concert with another RBR ligase, HOIL-1L (heme-oxidized IRP2 ubiquitin ligase 1 homolog), suggesting that other RBR ligases may function in collaboration, akin to two molecules of Parkin

Although these recent studies raise the possibility of a cooperative model for Parkin function, there remain several important caveats. One is that a cooperative model does not explain E2 interaction as the REP and Ubl remain attached at the E2 binding site. In addition, the absence of the Ubl-RING0 linker, removed to aid high-resolution crystallography, necessarily reduces interdomain flexibility. Further, the lack of a structure of pParkin makes it challenging to fully understand the role of Parkin phosphorylation, as phosphorylation of the Ubl and pUb binding releases the Ubl domain and greatly enhances E2~Ub interaction. Finally, Parkin mutants can rescue each other by functioning in trans, but does wild-type Parkin cooperate for ubiquitin transfer? To distinguish these models, rescuing Parkin mutants deficient in one part of the transfer cascade with others deficient in another would help decipher at which stage cooperation can occur.

Currently, the data supporting each model do not exclude the other model, and therefore, both modes of ubiquitin transfer—through conformational change or through cooperative binding—could occur. Parkin is mainly cytoplasmic, yet PINK1 resides at the mitochondria. Does this mean that Parkin is only activated at the mitochondria, or are there alternative mechanisms dependent on subcellular localization? For example, ubiquitin-interacting motifs on Parkin substrates have been shown to increase Parkin activity (*3*). Therefore, Parkin may employ distinct mechanisms of activation in different cellular contexts, which could include structural rearrangement and/or cooperation between molecules. Distinguishing between these models will facilitate understanding how and when Parkin recognizes substrates, and how ARPD-associated mutations disrupt Parkin function. Understanding Parkin's catalytic cycle will be important for targeting Parkin for therapeutic intervention. It will be fascinating to see what further conformations and mechanisms emerge as the understanding of the catalytic cycle of Parkin-mediated ubiquitin transfer deepens.

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Figure 1 Charging Parkin: Conformational change and cooperation models for Parkin function

For RBR ligases to function, they must engage the E2~Ub conjugate and transfer ubiquitin to the catalytic Cys in a transthiolation reaction prior to substrate lysine ubiquitination.

Models of Ubiquitin transfer to Cys^{431}.

Model 1: Conformational changes release the REP-Ubl and the RING2 for E2~Ub binding and exposure of Cys^{431}. This conformational change facilitates juxtaposition of the catalytic cysteines for transthiolation. Model 2: The REP-Ubl is destabilized and the ubiquitin binding site revealed. To bridge the distance for ubiquitin transfer to Cys^{431}, Parkin cooperates with another E2~Ub bound Parkin complex to load its Cys^{431} residue.

Ubl, ubiquitin-like domain (green); Ub, ubiquitin (purple); R0, RING0 (gray); R1, RING1 (red); R2, RING2 (cyan); IBR; in-between-ring domain (brown); REP, repressor of Parkin (yellow); pS65, phosphorylation of serine 65; PINK1, PTEN-induced putative kinase 1.